

CLAIMS

1.- Electroporation device for the permeabilization of cells (C) contained in a substrate (12) comprising signal generating means (3) for generating a stimulating signal (S(t)) applied by means of electrodes (6,7) to the substrate (12) wherein an electric field (E(t)) permeabilizing the cells membranes is induced;

the device being **characterized by comprising:**

- means for measuring, calculating and monitoring (15,16,23) the instantaneous value of the ratio (GT) of current (**ie**) flowing between said electrodes (6,7) and through the substrate (12) and the voltage (**Vp**) of the stimulating signal (S(t)) applied to the substrate (12) by means of said electrodes (6,7);

said device further comprising controlling means (100-170) for applying the stimulating signal in a controlled manner according to the waveform of an initial portion of the curve C_{GT} representing the ratio (GT) in successive instants after the beginning of the application of the stimulating signal (S(t)).

2.- Device as claimed in claim 1, wherein said controlling means (100-170) comprise timing means (110) for applying said stimulating signal for a predetermined period of time T_d and analysing the initial portion of the waveform of curve C_{GT} to detect a minimum value of

the curve C_{GT} within the interval $t = 0$ and $t = Td$.

3.- Device as claimed in claim 1, wherein said controlling means (100-170) calculate the slope of the waveform of curve C_{GT} after that a minimum in curve C_{GT} 5 has been reached.

4.- Device as claimed in claim 1, wherein said controlling means (100-170) comprise hazard detecting means (120) determining the instantaneous gradient (dG) of said ratio (GT) after a minimum has been reached in 10 said curve C_{GT} ;

 said controlling means further comprise first comparing means (130) for comparing the calculated instantaneous gradient dG with at least a reference value (dG_{ref1}) and selecting correcting means (140,145) 15 for performing an urgent correction to the stimulating signal $S(t)$ in order to avoid lesions, damages or irreversible alterations in said substrate (12).

5.- Device as claimed in claim 4, wherein said correcting means (140,145) decreases the voltage of the 20 stimulating signal $S(t)$ in order to prevent deterioration in the cells (C).

6.- Device as claimed in claim 1, wherein said controlling means (100-170) comprise slope determining means (150) calculating the average variation ΔG of said ratio (GT) in a time interval that is successive to the 25 instant Tm wherein a minimum in the curve (C_{GT}) has been

reached and that has a pre-determined time width;

said controlling means further comprising second comparing means (160) comparing the calculated average variation ΔG of said ratio (GT) with a reference 5 interval of ΔG values.

7.- Device as claimed in claim 6, wherein said second comparing means (160) performs the following functions:

- if the calculated average variation ΔG of said 10 ratio (GT) falls within the reference interval ($0 < \Delta G < \Delta G_{obb}$) continuing means (170) are selected;
- if the calculated average variation ΔG of said ratio (GT) falls outside the reference interval 15 and it is smaller than both limits delimiting the interval ($\Delta G < 0 < \Delta G_{obb}$) adjusting means (180) are selected; and
- if the calculated average variation ΔG of said ratio (GT) falls outside the reference interval 20 and it is greater than both limits delimiting the interval ($\Delta G > \Delta G_{obb} > 0$) correcting means (140) are selected.

8.- Device as claimed in claim 7, wherein said adjusting means (180) increase the voltage of the 25 stimulating signal in order to increase the value of the electric field $E(t)$ applied to the substrate (12); said

adjusting means (180) subsequently selecting said means for calculating and monitoring (15,16,23) the instantaneous value of the said ratio (GT) and said controlling means.

5 9.- Device as claimed in claim 7, wherein said continuing means (170) increase the voltage of the stimulating signal to an objective voltage V_{opt} in order to increase the value of the electric field $E(t)$ applied to the substrate (12) so that the value of said average
10 variation ΔG tends to an expected value ΔG_{obb} .

10. - Device as claimed in claim 1, wherein said controlling means (100-170) detects (125) a minimum of said initial portion of said curve and determining (126) the time T_m at which the minimum is reached.

15 11.- Device as claimed in claim 10, wherein third comparing means (127) are provided to compare the detected time T_m with threshold values T_{tmin} and T_{tmax} ; said third comparing means (127) performing the following operations:

- 20 - if the detected T_m occurs before T_{tmin} ($T_m < T_{tmin}$) then correcting means (140) are selected;
- if the detected T_m occurs after T_{tmax} ($T_m > T_{tmax}$), then adjusting means (180) are selected;
 and
- 25 - - if the detected T_m occurs between T_{tmin} and T_{tmax} , then continuing means (170) are selected.

12.- Use of a device as described in claim 1, to extract molecules from the living cells comprised in the substrate.

13.- Use of a device as described in claim 1, to 5 introduce molecules into living cells.

14.- Use of the device as claimed in claim 13, wherein said molecules comprise one of the following:

- ◆ a DNA or a RNA molecule containing regulatory sequences and sequence coding for therapeutic genes or genes of interest for biomedical or 10 biotechnological purposes;
- ◆ an oligonucleotide, (ribo- or deoxyribo-nucleotide, single or double strand, including the SiRNA), whether natural (phosphodiesters) or 15 modified (inside the backbone of the oligonucleotide, such as phosphosulfates, or at the extremities, by addition of groups to protect the oligonucleotides from digestion of nucleases);
- ◆ a protein or peptide, whether natural or genetically or chemically modified, extracted 20 from natural sources or obtained by synthesis, or a molecule simulating the structure of a protein or peptide, whatever its structure;
- ◆ a cytotoxic agent, in particular, the antibiotic 25 bleomycin or the cisplatinum;

◆ a penicillin; and
other pharmacological agents.